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Effect of inoculating the Awassi rumen fluid with its counterpart from Iraqi buffaloes on in vitro digestibility coefficient of barley straw and some characteristics of the rumen fluid

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The study was conducted at the College of Agricultural Engineering Sciences / University of Baghdad for the period from 3-30-2022 to 4-10-2022 with the aim of determining the extent of improvement or change in the rumen fluid of Awassi lambs after inoculation with Iraqi buffalo rumen fluid. 5 ratios of Iraqi buffalo rumen fluid were used. Which was collected immediately after slaughter and (0.1, 0.2, 0.4, 0.6, and 0.8) represented the treatments (T1, T2, T3, T4, and T5) were added to the rumen fluid of Awassi lambs in in vitro digestion tubes to estimate the in vitro digestibility coefficient of the dry and organic matter of barley straw, pH, and total number of microorganisms in rumen fluid. The results showed a highly significant improvement (P<0.01) for the treatments (T3, T4, and T5) in the in vitro digestibility coefficient of organic matter and dry matter compared to the two treatments (T1 and T2). The results also indicated a highly significant (P<0.01) superiority in the pH value of the two treatments (T2 and T4) with buffalo rumen fluid compared to the other treatments depending on the timing during which the pH was measured. In the same context, the treatments (T3, T4, and T5) outperformed the total number of microorganisms, and the results showed highly significant differences (P<0.01) for the two treatments (T3 and T4) in the total number of microorganisms compared to the treatments (T1, T2, and T5).From the results achieved above, we conclude that the two treatments (T3 and T4) for inoculation with buffalo rumen fluid were significantly superior in improving the in vitro digestibility coefficient of dry and organic matter and improving the characteristics of the rumen fluid in light of the results achieved above. This study is one of the first studies to improve the rumen environment in Awassi lambs through inoculation with different proportions of rumen fluid taken from Iraqi buffaloes after slaughter.

Keywords: Modifying, roughages, protozoa population, microbial digestion, Ruminants, awassi lambs, Iraqi buffalo rumen fluid, In vitro digestion, Barley straw, Organic matter, Microbial activity, Rumen environment.

INTRODUCTION

Modifying the rumen environment in ruminants has become a matter of concern to many scientists and researchers to improve the digestion coefficient in the rumen because of its great importance for ruminants. Research directed towards modifying and improving the rumen environment (Martin , 1998), with the aim of improving the microbial digestion in it to benefit from it more and thus increase digestion coefficients of roughages feeds with low nutritional value, and in view of the scarcity of roughages feeds and their low digestibility coefficients and nutritional value due to the binding of lignin with cellulose and hemicellulose and the limited digestive enzymes secreted by microorganisms in the rumen that are necessary to digest them and benefit from their nutritional elements (Hassan, 2004; Hassan and Mohammed, 2012). Therefore, several treatments were conducted that led to a significant improvement in rumen bacteria and the digestion coefficient of dry and organic matter of barley straw, such as treatment with urea and corn gluten (Hassan and Mohammed, 2017) and physical and chemical treatments (Hassan and Tawffek, 2009; Tawffek and Hassan, 2009), as well as treatment With probiotics (Alwaeli *et al.*, 2017) and treatment with probiotics and bread yeast (Al-Samaraae *et al.*, 2016). Accordingly, it has become necessary to study and modify the rumen environment and the metabolic reactions that accompany it, especially in small ruminants, to make the most of roughages and increase its digestibility coefficients, as some microbial treatments significantly improved the digestion coefficient of the dry and organic matter of barley

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straw (Al-Samraee, 2006 ; Hassan *et al.*, 2007 and Hashim *et al.*, 2008). Treatment with oyster mushrooms also improved the fermentation efficiency of silage made from barley straw (Hassan and Al-Samraee, 2015, 2020). Treating barley straw with the fungus (*Pleurotus ostreatus*) improved the in vitro digestibility coefficient of dry matter (DM) and organic matter (OM) (Hassan *et al.*, 2012). Treatment with the fungus (*Trichoderma harzianum*) also improved the in vitro digestibility of dry matter (DM) and organic matter (OM) of roughages (Al-Samaraee *et al.*, 2019). Recently, some studies have been conducted that have worked on direct feeding with rumen fluid to improve digestion and change the rumen environment and biology to some extent, as (Ribeiro *et al.*, 2017) indicated a change and improvement in the rumen microbiome in cattle in which the rumen was inoculated with rumen fluid taken from *American bison* immediately after slaughter, as well as successfully improving digestion. Here we are studying the inoculation of the rumen fluid of *Awassi lambs* with the rumen fluid of *Iraqi buffaloes* and its effect on the digestibility coefficient of dry and organic matter of barley straw and some characteristics of the rumen fluid. This is due to the important features that characterize the Iraqi buffalo, such as the unique and distinct environment of the rumen that distinguishes it from other ruminants, represented by the complex nature of the rumen and the high ability to convert roughages feed. In addition to the microorganisms that play an important role in the digestion process and the large numbers of large protozoa population that are highly efficient in digesting cellulose and nitrogen, in addition to the high rumen pH and concentration of ammonia and volatile fatty acids (VFA) in buffalo compared to their concentrations in the rumen of others ruminants (Mould *et al.*, 2005). All of these hypotheses are the subject of controversy and discussion by many researchers (Calabrò *et al.*, 2004 and Hall, 2001).

MATERIALS AND METHODS

This study was conducted at the College of Agricultural Engineering Sciences / University of Baghdad for the period from 3-30-2022 until 4-10-2022 to determine the extent of improvement or change in the rumen fluid of Awassi lambs after inoculation with its counterpart from Iraqi buffaloes. Fodder samples of barley straw were collected and withdrawn from them. Samples (500) milligrams for in vitro digestion. In this study, 5 treatments (T1, T2, T3, T4, and T5) with 3 replicates for each treatment, representing 5 inoculation ratios, by forming a mixture of the two liquids that were collected immediately after slaughter for each treatment. These 5 treatments were added to the digestion tubes containing 500 mg of dried and crushed samples of barley straw to estimate the in vitro digestibility coefficient of the organic matter, dry matter, pH, and to calculate the total number of microorganisms in the rumen fluid, by forming a mixture of the two liquids representing the five parameters, as follows:

- T1 :[(1ml) buffalo rumen fluid + (9 ml) sheep rumen fluid + barley straw samples].
- T2 :[(2ml) buffalo rumen fluid + (8 ml) sheep rumen fluid + barley straw samples].
- T3 :[(4 ml) buffalo rumen fluid + (6 ml) sheep rumen fluid + barley straw samples].
- T4 :[(6 ml) buffalo rumen fluid + (4 ml) sheep rumen fluid + barley straw samples].
- T5 :[(8 ml) buffalo rumen fluid + (2 ml) sheep rumen fluid + barley straw samples].

***Collection of rumen fluid*:** Awassi lambs and Iraqi buffalo rumen fluid were collected directly after slaughter from adult animals.

***In vitro tests and analyses*:** An in vitro digestion experiment was conducted to estimate the in vitro digestibility coefficient of organic dry matter of Iraqi wild cane according to the method of (Tilley and Terry, 1963). The pH of the rumen fluid was measured directly after collecting the fluid using a pH meter called Instruments Hanna (Nassar, 1971) and the numbers of anaerobic bacteria for each colony were calculated according to the method used in (Brewer and Allgeier, 1966; Van Milgen, 1993).

***Statistical Analysis*:** The statistical program Statistical Analysis System (SAS, 2012) was used to analyze the data to study the effect of barley straw and rates of inoculation with rumen fluid. It was applied in a completely randomized design (CRD), and the significant differences between the means were compared using the (Duncan, 1955) polynomial test according to the mathematical model below:

$Yij = \mu + Ti + eij$, since:
Yij: value of views.
μ: overall average of views.
Ti: Effect of percentages (T1, T2, T3, T4 and T5).
eij: effect of experimental error.

RESULTS AND DISCUSSION

***Effect of inoculating the rumen fluid of Awassi lambs with different proportions of the rumen fluid of Iraqi buffalo in the in vitro digestion of the dry matter and the organic matter of barley straw*:** The results in Table 1 indicate a highly significant improvement ($P<0.01$) in the in vitro digestibility coefficient of organic matter and dry matter of crushed barley straw for the treatments (T3, T4, and T5) as a result of the effect of inoculating the rumen fluid of Awassi lambs with different percentages of the rumen fluid of Iraqi buffaloes. With values reaching (27.610, 29.188, and 29.468) and (21.301, 22.590, and 23.746) for the organic matter and dry matter, respectively, compared to the two treatments (T1 and T2), with values reaching (21.122 and 23.865) and (13.200 and 16.375) for the organic matter and dry matter,

Table 1. Effect of inoculating the rumen fluid of Awassi lambs with different proportions of the rumen fluid of Iraqi buffalo in the in vitro digestion of the dry matter and the organic matter of barley straw.

Adjectives / Treatments	Means ± standard error	
	Dry matter-DM	**Organic matter-OM**
T1	13.200 ± 1.321b	21.122 ± 0.075c
T2	16.375 ± 0.752b	23.865 ±0.893b
T3	21.301 ± 0.502a	27.610 ± 0.846a
T4	22.590 ± 1.183a	29.188 ± 0.314a
T5	23.746 ± 1.400a	29.468 ± 1.278a
significant level	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01).
- T1 :[(1ml) buffalo rumen fluid + (9 ml) sheep rumen fluid + barley straw samples].
- T2 :[(2ml) buffalo rumen fluid + (8 ml) sheep rumen fluid + barley straw samples].
- T3 :[(4 ml) buffalo rumen fluid + (6 ml) sheep rumen fluid + barley straw samples].
- T4 :[(6 ml) buffalo rumen fluid + (4 ml) sheep rumen fluid + barley straw samples].
- T5 :[(8 ml) buffalo rumen fluid + (2 ml) sheep rumen fluid + barley straw samples].

respectively. At the same time, the organic matter digestibility coefficients were significantly superior (P≤0.01) compared to the dry matter digestion coefficients for the treatments (T3, T4, and T5) with the same values mentioned above. The in vitro digestibility coefficient of organic matter and dry matter for the two treatments (T4 and T5) with buffalo rumen fluid improved with values reaching (29.188 and 29.468) and (21.301 and 22.590) compared to the treatments (T1, T2, and T3). The in vitro digestibility coefficient of organic matter (OM) increased significantly (P<0.01) as a result of inoculation with Iraqi buffalo rumen fluid. The digestibility coefficient of dry and organic matter improved during inoculation with different concentrations and stages of rumen fluid contents for fifteen feed materials, (Bueno *et al.*, 2005). The in vitro dry matter digestibility coefficient of the rumen fluid mixture was improved when the *RUSITEC* rumen was cross-inoculated with rumen fluid from bison and cows compared to each fluid separately together, (Oss *et al.*, 2016). Inoculation with a mixture of rumen fluid from Holstein-Friesian and Charolaise calves within the same herd fed only rough fodder showed a superior ability to digest dry matter in the in vitro, (McDermott et al., 2020) and some microbial treatments significantly improved the digestibility coefficient of the dry and organic matter of barley straw, (Hashim *et al.*, 2008). Biological treatments such as inoculation and feeding with rumen fluid and contents lead to better rumen fermentations, (Sepriyadi, 2021), which in turn leads to an improvement in the digestibility coefficient of dry and organic matter.

According to the above results, it is clear that the digestibility coefficient of dry and organic matter and its effect on inoculation with rumen fluid also depends on the type of feed material, its content of dry and organic matter, and the speed of their decomposition, (Pamungkas *et al.*, 2006). The results of the above studies are consistent with the results of our current study.

***Effect of inoculation of lamb's rumen fluid with different proportions of buffalo's rumen fluid, feeding with barley straw and timing on the pH value*:** The results in Table 2 show a highly significant increase (P≤0.01) in the pH values as a result of the effect of pollination with different percentages of buffalo rumen fluid and feeding with barley straw. Highly significant differences (P≤0.01) in pH values were observed for the two inoculation treatments (T2 and T4) with buffalo rumen fluid, With values reaching 5.76 and 5.73 compared to the treatments (T1, T3, and T5) and values reaching 5.40, 5.50, and 5.39, respectively, after 6 hours of in vitro digestion at a temperature of 39°C. The results in Table (2) also show a highly significant increase (P≤0.01) in the pH values of the pollination treatment (T2) with a number of 6.84 compared to the pollination treatments (T1, T3, and T5) with numbers of 6.43, 6.59 and 6.40 for the three treatments, respectively, after 12 months. One hour of in vitro digestion. While it did not differ significantly with the inoculation treatment (T4). The results also show a highly significant increase (P≤0.01) in the pH values of the inoculation treatment (T4) with a number of 6.80 compared to the two inoculation treatments (T1 and T5) with numbers of 6.43 and 6.40, respectively, after 12 hours of in vitro digestion. While it did not differ significantly with the inoculation treatment (T4). In general, the pH values for all treatments and timings increased after 9 and 12 hours of in vitro incubation, with values significantly superior to their values after 6 hours of in vitro digestion (Table 2). The reason for the rise in pH values is due to the improvement in the digestibility coefficient of dry and organic matter and the moral superiority of rumen bacteria (Tables 1 and 3), which work in conjunction with pH, which in turn leads to an increase in rumen products of volatile fatty acids and other rumen by-products, which work to raise or Reducing rumen pH values. The degree of pH is a good indicator of the health of the rumen and the quality of digestion. The type of roughages can also determine the

Table 2. Effect of inoculation of lamb's rumen fluid with different proportions of buffalo's rumen fluid, feeding with barley straw and timing on the pH value.

Adjectives / Treatments	Means ± standard error		
	pH at Time-6	pH at Time-9	pH at Time-12
T1	5.400 ± 0.057 b	5.770 ± 0.063 b	6.433 ± 0.066 c
T2	5.766 ± 0.088 a	6.160 ± 0.094 a	6.840 ± 0.124 a
T3	5.506 ± 0.020 b	5.890 ± 0.026 b	6.590 ± 0.049 bc
T4	5.736 ± 0.020 a	6.100 ± 0.045 a	6.803 ± 0.052 ba
T5	5.393 ± 0.008 b	5.750 ± 0.028 b	6.406 ± 0.034 c
significant level	**	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01).
- T1 :[(1ml) buffalo rumen fluid + (9 ml) sheep rumen fluid + barley straw samples].
- T2 :[(2ml) buffalo rumen fluid + (8 ml) sheep rumen fluid + barley straw samples].
- T3 :[(4 ml) buffalo rumen fluid + (6 ml) sheep rumen fluid + barley straw samples].
- T4 :[(6 ml) buffalo rumen fluid + (4 ml) sheep rumen fluid + barley straw samples].
- T5 :[(8 ml) buffalo rumen fluid + (2 ml) sheep rumen fluid + barley straw samples].

Table 3. The effect of inoculating the rumen fluid of Awassi lambs with different proportions of the rumen fluid of freshly slaughtered Iraqi buffalo and barley straw, concentration and timing on the total number of bacteria.

Adjectives / Treatments	Mean total number of bacteria/ CFU-Log ± standard error	
	After 4 hours of digestion	After 48 hours of digestion
T1	11.031 ± 0.026 b	11.231 ± 0.054 b
T2	11.238 ± 0.084 ab	11.337 ± 0.053 ba
T3	11.279 ± 0.064 a	11.396 ± 0.017 a
T4	11.316 ± 0.032 a	11.417 ± 0.013 a
T5	11.245 ± 0.096 b	11.386 ± 0.042 a
significant level	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01), NS: not significant.
- T1 :[(1ml) buffalo rumen fluid + (9 ml) sheep rumen fluid + barley straw samples].
- T2 :[(2ml) buffalo rumen fluid + (8 ml) sheep rumen fluid + barley straw samples].
- T3 :[(4 ml) buffalo rumen fluid + (6 ml) sheep rumen fluid + barley straw samples].
- T4 :[(6 ml) buffalo rumen fluid + (4 ml) sheep rumen fluid + barley straw samples].
- T5 :[(8 ml) buffalo rumen fluid + (2 ml) sheep rumen fluid + barley straw samples].

degree of pH, which is mainly affected by the feed and food materials that form acids and the ratio of concentrated feed to roughages feed. We would like to point out that the rise in pH values during in vitro digestion is a normal matter compared to digestion inside the rumen of a ruminant animal, due to the large production and circulation of saliva during digestion inside the rumen of the ruminant and because the saliva contains regulators that work in Simultaneously with the pH of the rumen, and the rumen is constantly working to absorb feed particles. It creates or removes acidity by passing it into the intestine. The results agreed with (Wang *et al.*, 2016) who indicated an increase in the pH values of the rumen fluid in the group of lambs that were inoculated with the rumen fluid of adult ewes under two different feeding systems, The results are also consistent with, (Zhong *et al.*, 2014), who indicated that rumen pH, ammonia N concentrations, and total VFA among weaned lambs inoculated with rumen fluid were not affected, but differed significantly between sampling times. While the results did not agree with (McDermott *et al.*, 2020) who indicated a decrease in the pH values. During in vitro digestion as a result of cross-inoculation of rumen fluid taken from Holstein-Friesian and Charolaise calves immediately after slaughter. We conclude from the pH values.

The effect of inoculating the rumen fluid of Awassi lambs with different proportions of the rumen fluid of freshly slaughtered Iraqi buffalo and barley straw, concentration and timing on the total number of bacteria: The results in Table 3 show a highly significant superiority (P≤0.01) for the two treatments (T3 and T4) in the total number of rumen microorganisms after 4 hours of in vitro digestion as a result of inoculation with buffalo rumen fluid, with total numbers of 11,279 and 11,316, respectively, compared to the inoculation treatment (T1) in total numbers of microorganisms. It reached 11,031, while it did not differ significantly when compared to the two vaccination treatments (T2 and T5), with total numbers reaching 11,238 and 11,245, respectively. The results in the same table also showed a highly significant superiority (P≤0.01) in the total number of rumen

microorganisms for the buffalo rumen fluid inoculation treatments (T3, T4, and T5) after 48 hours of in vitro digestion with total numbers reaching 11,396, 11,417 and 11,386, respectively, compared to the treatment Inoculation (T1) had a total number of 11,231, while it did not differ significantly when compared to the treatment (T2) with a total number of 11,337, respectively. The significant increase in the total number of rumen microorganisms may be attributed to the increase in the pH values of the rumen and the digestion coefficients of dry and organic matter, as in Tables (1 and 2) of the current study, which enhances the activity of microorganisms in general, in addition to the relative abundance of microorganisms provided by inoculation with rumen fluid. Buffalo, as fiber-digesting bacteria, grows when the pH is between (6 - 6.8). The results are consistent with (Hashim et al., 2008), as microbial treatments improved fermentation and rumen microbiota. The results are also consistent with (Ribeiro et al., 2017), as the rumen microbiota became more diverse and showed a higher balance as a result of rumen fluid transfers, in addition to insemination and feeding with rumen fluid. And rumen contents led to better rumen fermentations (Sepriyadi, 2021) which in turn improved the rumen microbial yield. Microbiological food additives rely on selected bacterial or yeast cultures and they affect rumen fermentations and improve animal health by modifying the gastrointestinal bacteria (Tavendale et al., 2005). (Shabi et al., 1998) explained that the microbial yield in the rumen depends on many factors such as the availability of carbohydrates and nitrogen in the rumen and the pH of the rumen (Finlayson, 1986).

***Conclusions*:** From the results achieved above in tables (1, 2 and 3), we conclude that the rates of inoculation with buffalo rumen fluid in the third, fourth and fifth treatments achieved the best results in improving the coefficient of digestion of the organic and dry materials and the modification of the rumen environment compared to the first and second treatments. In this context, we recommend conducting expanded experiments that include a larger number of replicates and experimental treatments for inoculation with rumen fluid, as well as the inclusion of more periods of timing for drawing samples of rumen fluid and the microbial community, and the inclusion of in vitro protein digestion laboratories to find out the effect of inoculation on the protein digestion coefficient and its reflection on the characteristics of the rumen environment.

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